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EXAMINER	
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ART UNIT	**PAPER NUMBER**
1637	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	01/10/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	10/767,779	LEAMON ET AL.	
	Examiner	Art Unit	
	Samuel Woolwine	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *04 October 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-84* is/are pending in the application.

4a) Of the above claim(s) *10-39,41-46 and 65-71* is/are withdrawn from consideration.

5)☒ Claim(s) *1-9 and 73-75* is/are allowed.

6)☒ Claim(s) *40,47-64,72 and 76-84* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *8/2/2004*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

Applicant's election with traverse of Group I, claims 1-9, 40, 47-64 and 72-84 in the reply filed on 10/4/2006 is acknowledged. The traversal is on the ground(s) that there would not be a burden of search, as the two groups have both been classified in class 435, and as the two searches would be partially overlapping. This is not found persuasive because the mere fact that two inventions fall within the same class does not mean searching both inventions would not be a burden.

Furthermore, the fact that two searches would be partially overlapping does not mean the two searches would be co-extensive, contrary to the assertion of Applicant. The assertion that the claims "represent a web of knowledge and continuity of effort" is not a basis upon which restriction is or is not required, nor is the matter of expense. The requirements for a proper restriction are a showing of patentable distinction and serious burden of search, which were detailed in the previous Office action.

The requirement is still deemed proper and is therefore made FINAL. Claims 10-39, 41-46 and 65-71 are withdrawn from further consideration.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 47-51 and 76-84 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 47 and 76 recite the limitation "the

well" (line 9 in each of the claims). There is insufficient antecedent basis for this limitation in the claims. It will be assumed for purposes of examination that "the well" refers to one of the "at least 10,000 discrete reaction chambers".

Claim 80 is further rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 80 recites the limitation "the amplifying step" in reference to claim 76. There is insufficient antecedent basis for this limitation in the claim.

Claim 64 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 64 recites the limitation "said PPi—detection enzyme" in reference to claim 63. There is insufficient antecedent basis for this limitation in the claim.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

Claims 40, 52, 53, 59-61 and 72 are rejected under 35 U.S.C. 102(e) as being anticipated by Weiner et al (US 2005/0009022, filing date July 8, 2002, priority date July 6, 2001).

The applied reference has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art under 35 U.S.C. 102(e). This rejection under 35 U.S.C. 102(e) might be overcome either by a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of this application and is thus not the invention "by another," or by an appropriate showing under 37 CFR 1.131.

With regard to claim 40, Weiner teaches *a method for delivering a bioactive agent to an array, comprising dispersing over the array a plurality of mobile solid supports, each mobile solid support having at least one reagent immobilized thereon, wherein the reagent is suitable for use in a nucleic acid sequencing reaction* (see paragraph [0124]: "A bioactive agent could be delivered to the array, by dispersing over the array a plurality of mobile solid supports, each mobile solid support having at least one reagent immobilized thereon, wherein the reagent is suitable for use in a nucleic acid sequencing reaction"),

where the array comprises a planar surface with a plurality of reaction chambers disposed thereon (see paragraph [0058]: "planar array of reaction sites").

With regard to claim 52, Weiner teaches *a method for delivering nucleic acid sequencing enzymes to an array* (see paragraph [0125]: "array can also include a population of mobile solid supports disposed in the reaction chambers, each mobile

solid support having one or more bioactive agents (such as a nucleic acid or a sequencing enzyme) attached thereto"), *said array having a planar surface with a plurality of cavities thereon, each cavity forming an analyte reaction chamber* (see paragraph [0058]: "planar array of reaction sites"; see paragraph [0125]: "we prefer the diameter of the mobile solid support to be between 0.01 to 0.1 times the width of each cavity"), *wherein the reaction chambers have a center to center spacing of between 20 to 100 μm* (see paragraph [0114]: "array is typically constructed to have reaction chambers with a center-to-center spacing between 5 to 200 μm"); *the method comprising dispersing over the array a plurality of mobile solid supports having one or more nucleic acid sequencing enzymes immobilized thereon* (see paragraph [0125]: "array can also include a population of mobile solid supports disposed in the reaction chambers, each mobile solid support having one or more bioactive agents (such as a nucleic acid or a sequencing enzyme) attached thereto"), *such that a plurality of the reaction chambers contain at least one mobile solid support* (see paragraph [0125]: for example, "where 50% to 100% of the reaction chambers can have a mobile solid support having at least one reagent immobilized thereon").

With regard to claim 53, see paragraph [0126]: "mobile solid support typically has at least one reagent immobilized thereon. For the embodiments relating to pyrosequencing reactions or more generally to ATP detection, the reagent may be a polypeptide with sulfurylase or luciferase activity, or both".

With regard to claim 59, Weiner teaches *a method for sequencing a nucleic acid, the method comprising: (a) providing a plurality of single-stranded nucleic acid*

templates disposed within a plurality of cavities (see paragraph [0123]: "those reaction chambers that contain a nucleic acid (not all reaction chambers in the array are required to) contain only a single species of nucleic acid... In one embodimant, the nucleic acid is single stranded") *on a planar surface* (see paragraph [0058]: "planar array of reaction sites"), *each cavity forming an analyte reaction chamber* (paragraph [0123]: "each cavity or reaction chamber of the array contains reagents for analyzing a nucleic acid"), *wherein the reaction chambers have a center to center spacing of between 20 to 100 μm* (see paragraph [0114]: "array is typically constructed to have reaction chambers with a center-to-center spacing between 5 to 200 μm") *and the planar surface has at least 10,000 reaction chambers* (paragraph [0114]); *(b) performing a pyrophosphate based sequencing reaction simultaneously on all reaction chambers* (see paragraphs [0143]-[0152]) *by annealing an effective amount of a sequencing primer to the nucleic acid templates* (see paragraph [0035]: "each reaction mixture comprising a template-directed nucleotide polymerase and a single-stranded polynucleotide template hybridized to a complementary oligonucleotide primer strand") *and extending the sequencing primer with a polymerase and a predetermined nucleotide triphosphate to yield a sequencing product and, if the predetermined nucleotide triphosphate is incorporated onto the 3' end of said sequencing primer, a sequencing reaction byproduct* (see paragraph [0149]); *and (c) identifying the sequencing reaction byproduct, thereby determining the sequence of the nucleic acid in each reaction chamber* (see paragraph [0152]).

With regard to claim 60, see paragraphs [0149] and [0150].

With regard to claim 61, paragraph [0126]: "mobile solid support typically has at least one reagent immobilized thereon. For the embodiments relating to pyrosequencing reactions or more generally to ATP detection, the reagent may be a polypeptide with sulfurylase or luciferase activity, or both".

With regard to claim 72, Weiner teaches *a method for sequencing a nucleic acid, the method comprising: (a) providing a plurality of single-stranded nucleic acid templates in an array* (paragraph [0123]: "those reaction chambers that contain a nucleic acid (not all reaction chambers in the array are required to) contain only a single species of nucleic acid... In one embodimant, the nucleic acid is single stranded" and paragraph [0040]: "the reaction site is comprised of a template-directed nucleotide polymerase and a heterogenous population of single stranded templates hybridized to complementary oligonucleotide primer strands") *having at least 50,000 discrete reaction sites* (paragraph [0114])

(b) contacting the nucleic acid templates with reagents necessary to perform a pyrophosphate-based sequencing reaction coupled to light emission (paragraph [0041]);

(c) detecting the light emitted from a plurality of reaction sites on respective portions of an optically sensitive device (paragraph [0042]);

(d) converting the light impinging upon each of said portions of said optically sensitive device into an electrical signal which is distinguishable from the signals from all of said other reaction sites (paragraph [0041]);

(e) determining the sequence of the nucleic acid templates based on light emission for each of said discrete reaction sites from the corresponding electrical signal (paragraph [0041]).

Claims 47-51 and 62-64 are rejected under 35 U.S.C. 102(e) as being anticipated by Rothberg et al (US 2003/0068629 filing date March 21, 2002).

The applied reference has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art under 35 U.S.C. 102(e). This rejection under 35 U.S.C. 102(e) might be overcome either by a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of this application and is thus not the invention "by another," or by an appropriate showing under 37 CFR 1.131.

With regard to claim 47, Rothberg teaches *a method for carrying out separate parallel common reactions in an aqueous environment, comprising: (a) delivering a fluid containing at least one reagent to an array* (see paragraph [0030]), *wherein the array comprises a substrate comprising at least 10,000 discrete reaction chambers* (see paragraph [0018]), *each reaction chamber adapted to contain analytes* (see paragraph [0024]), *and wherein the reaction chambers have a volume of between 10 to 150 pL* (Rothberg teaches each chamber can be smooth-walled and cylindrical in shape with a planar bottom (paragraph [0018]), and that the depth can be between 0.25 and 5 times the width (paragraph [0020]), and that the width can be between 0.3 and 100 μm (paragraph [0017]). Since the volume of a cylinder is $\pi r^2 h$, where r is the radius, or half

the diameter (width), and h is the height (depth), Rothberg teaches reaction chambers with volumes ranging from 5.3×10^{-3} μm^3 (for 0.3 μm wide wells with 0.25× depth) to 3.93×10^{6} μm^3 (for 100 μm wide wells with 5× depth). Converting μm^3 to pL gives a range of 5.3×10^{-3} pL to 3930 pL.) *and containing a starting material that is capable of reacting with the reagent* (see paragraph [0030]), *each of the reaction chambers being dimensioned such that when the fluid is delivered into each reaction chamber, the diffusion time for the reagent to diffuse out of the well exceeds the time required for the starting material to react with the reagent to form a product* (see paragraph [0030]); *and (b) washing the fluid from the array in the time period (i) after the starting material has reacted with the reagent to form a product in each reaction chamber but (ii) before the reagent delivered to any one reaction chamber has diffused out of that reaction chamber into any other reaction chamber* (see paragraph [0030]).

With regard to claim 48, see paragraph [0030].

With regard to claim 49, see paragraph [0030].

With regard to claim 50, see paragraph [0030].

With regard to claim 51, see paragraph [0030].

With regard to claim 62, Rothberg teaches *a method of determining the base sequence of a plurality of nucleotides on an array* (see paragraph [0133]), *the method comprising: (a) providing at least 10,000 DNA templates* (paragraph [0018], which teaches up to 20 million reaction chambers and see abstract: "permitting the simultaneous sequencing of a very large number (>10,000) of different oligonucleotides"),

each separately disposed within a plurality of cavities (paragraph [0133] and paragraph [0024]: "those reaction chambers that contain a nucleic acid (not all reaction chambers in the array are required to) contain only a single species of nucleic acid"),

each cavity forming an analyte reaction chamber (paragraph [0133]),

wherein the reaction chambers have a center to center spacing of between 20 to 100 μm (paragraph [0133]),

and a volume of between 10 to 150 pL (see discussion of this limitation for claim 47 above);

wherein (b) adding an activated nucleotide 5'-triphosphate precursor of one known nitrogenous base to a reaction mixture in each reaction chamber (paragraph [0133]),

each reaction mixture comprising a template-directed nucleotide polymerase and a single-stranded polynucleotide template hybridized to a complementary oligonucleotide primer strand at least one nucleotide residue shorter than the templates to form at least one unpaired nucleotide residue in each template at the 3'-end of the primer strand, under reaction conditions which allow incorporation of the activated nucleoside 5'-triphosphate precursor onto the 3'-end of the primer strands, provided the nitrogenous base of the activated nucleoside 5'-triphosphate precursor is complementary to the nitrogenous base of the unpaired nucleotide residue of the templates (paragraph [0133]);

(c) detecting whether or not the nucleoside 5'-triphosphate precursor was incorporated into the primer strands in which incorporation of the nucleoside 5'-

triphosphate precursor indicates that the unpaired nucleotide residue of the template has a nitrogenous base composition that is complementary to that of the incorporated nucleoside 5'-triphosphate precursor (paragraph [0133]);

and (d) sequentially repeating steps (b) and (c), wherein each sequential repetition adds and, detects the incorporation of one type of activated nucleoside 5'-triphosphate precursor of known nitrogenous base composition (paragraph [0133]);

and (e) determining the base sequence of the unpaired nucleotide residues of the template in each reaction chamber from the sequence of incorporation of said nucleoside precursors (paragraph [0133]).

With regard to claim 63, Rothberg teaches *a method of identifying the base in a target position in a DNA sequence of template DNA* (paragraph [0137]), *wherein: (a) at least 10,000 separate DNA templates* (paragraph [0018], which teaches up to 20 million reaction chambers and see abstract: "permitting the simultaneous sequencing of a very large number (>10,000) of different oligonucleotides") *are separately disposed within a plurality of cavities on a planar surface* (paragraph [0137]; paragraph [0133] and paragraph [0024]: "those reaction chambers that contain a nucleic acid (not all reaction chambers in the array are required to) contain only a single species of nucleic acid"), *each cavity forming an analyte reaction chamber* (paragraph [0137]), *wherein the reaction chambers have a center to center spacing of between 20 to 100 μm* (paragraph [0137]), *said DNA being rendered single stranded either before or after being disposed in the reaction chambers* (paragraph [0137]), *(b) an extension primer is provided which hybridizes to said immobilized single-stranded DNA at a position immediately adjacent*

to said target position (paragraph [0137]); *(c) said immobilized single-stranded DNA is subjected to a polymerase reaction in the presence of a predetermined deoxynucleotide or dideoxynucleotide, wherein if the predetermined deoxynucleotide or dideoxynucleotide is incorporated onto the 3' end of said sequencing primer then a sequencing reaction byproduct is formed* (paragraph [0137]); *and (d) identifying the sequencing reaction byproduct, thereby determining the nucleotide complementary to the base at said target position in each of the 10,000 DNA templates* (paragraph [0137]; paragraph [0018], which teaches up to 20 million reaction chambers and see abstract: "permitting the simultaneous sequencing of a very large number (>10,000) of different oligonucleotides").

With regard to claim 64, see paragraph [0137].

Claims 76, 80-82 and 84 are rejected under 35 U.S.C. 102(e) as being anticipated by Chee et al (US 2003/0108867 cited reference A95 on the IDS of 8/2/2004).

With regard to claim 76, Chee teaches *a method for sequencing nucleic acids comprising:*

(a) fragmenting large template nucleic acid molecules to generate a plurality of fragmented nucleic acids (see paragraph [0025]: "As will be appreciated by those in the art, the complementary target sequence may take many forms. For example, it may be contained within a larger nucleic acid sequence, i.e. all or part of a gene or mRNA, a restriction fragment of a plasmid or genomic DNA, among others");

(b) attaching one strand of a plurality of the fragmented nucleic acids individually to beads to generate single stranded nucleic acids attached individually to beads (see paragraph [0088]: "Only a single type of capture probe should be bound to a bead..."; and see figure 1, which illustrates a single stranded target attached to a bead);

(c) delivering a population of the single stranded fragmented nucleic acids attached individually to beads (see paragraph [0009]: "hybridization complexes are attached to microspheres distributed on the surface"; and see figure 1)

to an array of at least 10,000 reaction chambers (see paragraph [0109]: "wells or small depressions in the substrate that can retain the beads"; see figure 1; see paragraph [0104]: "Arrays containing from about 2 different nucleic acids (e.g. different beads, when beads are used) to many billions can be made...")

on a planar surface ("Generally the substrate is flat (planar)..."; see paragraph 0107])

wherein a plurality of the wells comprise no more than a one [*sic*] *bead with on* [*sic*] *single stranded fragmented nucleic acid* (see paragraph [0110]: "the surface of the substrate may be modified such that discrete sites are formed that can only have a single associated bead"; see also paragraph [0088]: "Only a single type of capture probe should be bound to a bead...")

(d) performing a sequencing reaction simultaneously on a plurality of the reaction chambers (see for example paragraph [0033]: "pyrosequencing on arrays"; see also paragraph [0043]: "A substrate comprising microspheres containing the target sequences and extension primers, forming hybridization complexes, is dipped or

contacted with a volume (reaction chamber or well) comprising a single type of dNTP, an extension enzyme, and the reagents and enzymes necessary to detect PPi").

With regard to claim 80, see paragraph [0019]: "the present invention provides methods of sequencing target nucleic acids in sample solutions...the sample may be the products of an amplification reaction...such as PCR amplification reaction".

With regard to claim 81, see paragraph [0043]: "A substrate comprising microspheres containing the target sequences and extension primers, forming hybridization complexes, is dipped or contacted with a volume (reaction chamber or well) comprising a single type of dNTP, an extension enzyme, and the reagents and enzymes necessary to detect PPi").

With regard to claim 82, Chee teaches:

(f) annealing an effective amount of a sequencing primer to the nucleic acid templates (see paragraph [0034]: "the sequencing primer is added and hybridized to the target sequence")

and extending the sequencing primer with a polymerase (see paragraph [0043]: "extension enzyme"; see also paragraph [0037]: "an extension enzyme, generally a DNA polymerase")

and a predetermined nucleotide triphosphate to yield a sequencing product and, if the predetermined nucleotide triphosphate is incorporated onto the 3' end of said sequencing primer, a sequencing byproduct (see paragraph [0043]: "If the dNTP is complementary to the base of the target portion of the target sequence adjacent to the

extension primer, the dNTP is added, releasing PPi"; it was well known in the art that DNA polymerases add dNTPs to the 3' end);

and (g) identifying the sequencing reaction byproduct, thereby determining the sequence of the nucleic acid in a plurality of reaction chambers (see paragraph [0043]: "releasing PPi and generating detectable light" and "This process is repeated to generate a readout of the sequence of the target sequence").

With regard to claim 84, Chee teaches *reaction chambers are cavities formed by etching on end of a fiber optic bundle.* See paragraph [0112]: "wells are made in a terminal or distal end of a fiber optic bundle comprising individual fibers...the cores of the individual fibers are etched".

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 40, 52-55, 59-63 and 77 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chee et al (US 2003/0108867 cited reference A95 on the IDS of 8/2/2004) in view of Wolber et al (USPN 6,284,465, issued 9/4/2001, filed 4/15/1999).

With regard to claim 40, Chee teaches *a method for delivering a bioactive agent to an array, comprising dispersing over the array a plurality of mobile solid supports* ("hybridization complexes are attached to microspheres distributed on the surface"; see paragraph [0009]),

each mobile solid support having at least one reagent immobilized thereon, wherein the reagent is suitable for use in a nucleic acid sequencing reaction ("hybridization complex"; see paragraph [0009]),

where the array comprises a planar surface ("Generally the substrate is flat (planar)..."; see paragraph 0107])

with a plurality of reaction chambers disposed thereon ("wells or small depressions in the substrate that can retain the beads"; see paragraph [0109] and see figure 1).

With regard to claim 52, Chee teaches *a method for delivering nucleic acid sequencing enzymes to an array, said array having a planar surface* ("Generally the substrate is flat (planar)..."; see paragraph 0107])

with a plurality of cavities thereon, each cavity forming an analyte reaction chamber ("wells or small depressions in the substrate that can retain the beads"; see paragraph [0109] and see figure 1),

the method comprising dispersing over the array a plurality of mobile solid supports having one or more nucleic acid sequencing enzymes immobilized thereon (see for example paragraphs [0057] where Chee teaches attaching the secondary enzymes required for generating the signal in pyrosequencing to beads, and see for example paragraph [0153]: "a substrate comprising the surface with the discrete sites is immersed into a solution comprising the particles"),

such that a plurality of the reaction chambers contain at least one mobile solid support (see paragraph [0158]: Chee teaches that there may be some sites (a *plurality*) on the array that contain more than one bead (which is *at least one*)).

With regard to claim 53, see paragraph [0057] where Chee teaches attaching the secondary enzymes required for generating the signal in pyrosequencing to beads, and see paragraph [0040] where Chee teaches the secondary enzymes for generating the signal in pyrosequencing are sulfurylase and luciferase.

With regard to claim 54, Chee teaches *a method for delivering a plurality of nucleic acid templates to an array* ("hybridization complexes are attached to microspheres distributed on the surface"; see paragraph [0009]),

said array having a planar surface ("Generally the substrate is flat (planar)..."; see paragraph [0107])

with a plurality of cavities thereon, each cavity forming an analyte reaction chamber ("wells or small depressions in the substrate that can retain the beads"; see paragraph [0109] and see figure 1),

the array having at least 10,000 reaction chambers (see paragraph [0104]: "Arrays containing from about 2 different nucleic acids (e.g. different beads, when beads are used) to many billions can be made...");

the method comprising dispersing over the array a plurality of mobile solid supports ("hybridization complexes are attached to microspheres distributed on the surface"; see paragraph [0009]),

each mobile solid support having no more than a single species of nucleic acid template immobilized thereon (see paragraph [0088]: "Only a single type of capture probe should be bound to a bead..."),

the dispersion causing no more than one mobile solid support to be disposed within any one reaction chamber (see paragraph [0110]: "the surface of the substrate may be modified such that discrete sites are formed that can only have a single associated bead").

With regard to claim 55, see paragraph [0024], where Chee teaches the nucleic acids may be either single or double stranded.

With regard to claim 59, Chee teaches *a method for sequencing a nucleid acid, the method comprising: (a) providing a plurality of single-stranded nucleic acid templates disposed* ("hybridization complexes are attached to microspheres distributed on the surface"; see paragraph [0009])

within a plurality of cavities ("wells or small depressions in the substrate that can retain the beads"; see paragraph [0109] and see figure 1)

on a planar surface ("Generally the substrate is flat (planar)..."; see paragraph [0107])

each cavity forming an analyte reaction chamber ("wells or small depressions in the substrate that can retain the beads"; see paragraph [0109] and see figure 1),

wherein the planar surface has at least 10,000 reaction chambers (see paragraph [0104]: "Arrays containing from about 2 different nucleic acids (e.g. different beads, when beads are used) to many billions can be made...");

(b) performing a pyrophosphate based sequencing reaction simultaneously on all reaction chambers (see for example paragraph [0033]: "pyrosequencing on arrays"; see also paragraph [0043]: "A substrate comprising microspheres containing the target sequences and extension primers, forming hybridization complexes, is dipped or contacted with a volume (reaction chamber or well) comprising a single type of dNTP, an extension enzyme, and the reagents and enzymes necessary to detect PPi")

by annealing an effective amount of a sequencing primer to the nucleic acid templates (see paragraph [0034]: "the sequencing primer is added and hybridized to the target sequence")

and extending the sequencing primer with a polymerase (see paragraph [0043]: "extension enzyme"; see also paragraph [0037]: "an extension enzyme, generally a DNA polymerase")

and a predetermined nucleotide triphosphate to yield a sequencing product and, if the predetermined nucleotide triphosphate is incorporated onto the 3' end of said sequencing primer, a sequencing byproduct (see paragraph [0043]: "If the dNTP is complementary to the base of the target portion of the target sequence adjacent to the extension primer, the dNTP is added, releasing PPi"; it was well known in the art that DNA polymerases add dNTPs to the 3' end);

and (c) identifying the sequencing reaction byproduct, thereby determining the sequence of the nucleic acid in each reaction chamber (see paragraph [0043]: "releasing PPi and generating detectable light" and "This process is repeated to generate a readout of the sequence of the target sequence").

With regard to claim 60, see paragraph [0043]: "PPi" and see paragraph [0040] where Chee teaches the secondary enzymes for generating the signal in pyrosequencing are sulfurylase and luciferase.

With regard to claim 61, see for example paragraphs [0057] where Chee teaches attaching the secondary enzymes required for generating the signal in pyrosequencing to beads, and see for example paragraph [0153]: "a substrate comprising the surface

with the discrete sites is immersed into a solution comprising the particles". Additionally, see paragraph [0040] where Chee teaches the secondary enzymes for generating the signal in pyrosequencing are sulfurylase and luciferase.

With regard to claim 62, Chee teaches *a method of determining the base sequence of a plurality of nucleotides on an array, the method comprising:*

(a) providing at least 10,000 DNA templates (see paragraph [0009]: "hybridization complexes are attached to microspheres distributed on the surface"; and see paragraph [0104]: "Arrays containing from about 2 different nucleic acids (e.g. different beads, when beads are used) to many billions can be made..."), *each separately disposed* (see paragraph [0110]: "the surface of the substrate may be modified such that discrete sites are formed that can only have a single associated bead") *within a plurality of cavities on a planar surface* ("Generally the substrate is flat (planar)..."; see paragraph [0107]), *each cavity forming an analyte reaction chamber* ("wells or small depressions in the substrate that can retain the beads"; see paragraph [0109] and see figure 1),

(b) adding an activated nucleotide 5'-triphosphate precursor of one known nitrogenous base to a reaction mixture in each reaction chamber (see for example paragraph [0043]: "A substrate comprising microspheres containing the target sequences and extension primers, forming hybridization complexes, is dipped or contacted with a volume (reaction chamber or well) comprising a single type of dNTP, an extension enzyme, and the reagents and enzymes necessary to detect PPi"; there is

no definition or explanation in the instant specification to distinguish "*an activated nucleotide 5'-triphosphate precursor*" from a dNTP),

each reaction mixture comprising a template-directed nucleotide polymerase (see paragraph [0043]: "extension enzyme" and see paragraph [0037]: "an extension enzyme, generally a DNA polymerase") *and a single-stranded polynucleotide template hybridized to a complementary oligonucleotide primer strand at least one nucleotide shorter than the templates to form at least one unpaired nucleotide residue in each template at the 3'-end of the primer strand* (see for example paragraph [0043]: "A substrate comprising microspheres containing the target sequences and extension primers, forming hybridization complexes..." and see figure 1), *under reaction conditions which allow incorporation of the activated nucleoside 5'-triphosphate precursor onto the 3'-end of the primer strands* (see paragraph [0043]: "If the dNTP is complementary to the base of the target portion of the target sequence adjacent to the extension primer, the dNTP is added, releasing PPi"; it was well known in the art that DNA polymerases add dNTPs to the 3' end), *provided the nitrogenous base of the activated nucleoside 5'-triphosphate precursor is complementary to the nitrogenous base of the unpaired nucleotide residue of the templates;*

(c) detecting whether or not the nucleoside 5'-triphosphate precursor was incorporated into the primer strands in which incorporation of the nucleoside 5'-triphosphate precursor indicates that the unpaired nucleotide residue of the template has a nitrogenous base composition that is complementary to that of the incorporated nucleoside 5'-triphosphate precursor (see paragraph [0043]: "releasing PPi and

generating detectable light" and "This process is repeated to generate a readout of the sequence of the target sequence");

and (d) sequentially repeating steps (b) and (c), wherein each sequential repetition adds and detects the incorporation of one type of activated nucleoside 5'-triphosphate precursor of known nitrogenous base composition (see paragraph [0043]: "releasing PPi and generating detectable light" and "This process is repeated to generate a readout of the sequence of the target sequence");

and (e) determining the base sequence of the unpaired nucleotide residues of the template in each reaction chamber from the sequence of incorporation of said nucleoside precursors (see paragraph [0043]: "releasing PPi and generating detectable light" and "This process is repeated to generate a readout of the sequence of the target sequence").

With regard to claim 63, Chee teaches *a method of identifying the base in a target position in a DNA sequence of template DNA, wherein:*

(a) at least 10,000 separate DNA templates (see paragraph [0009]: "hybridization complexes are attached to microspheres distributed on the surface"; and see paragraph [0104]: "Arrays containing from about 2 different nucleic acids (e.g. different beads, when beads are used) to many billions can be made...") *are separately disposed* (see paragraph [0110]: "the surface of the substrate may be modified such that discrete sites are formed that can only have a single associated bead") *within a plurality of cavities on a planar surface* ("Generally the substrate is flat (planar)..."; see paragraph [0107]), *each cavity forming an analyte reaction chamber* ("wells or small depressions in the

substrate that can retain the beads"; see paragraph [0109] and see figure 1), *said DNA being rendered single stranded either before or after being disposed in the reaction chambers* (see paragraph [0024], where Chee teaches the nucleic acids may be either single or double stranded),

(b) an extension primer is provided which hybridizes to said immobilized single-stranded DNA at a position immediately adjacent to said target position (see for example figure 1A and paragraph [0015]);

(c) said immobilized single-stranded DNA is subjected to a polymerase reaction in the presence of a predetermined deoxynucleotide or dideoxynucleotide, wherein if the predetermined deoxynucleotide or dideoxynucleotide is incorporated onto the 3' end of said sequencing primer then a sequencing reaction byproduct is formed (see paragraph [0043]: "If the dNTP is complementary to the base of the target portion of the target sequence adjacent to the extension primer, the dNTP is added, releasing PPi"; it was well known in the art that DNA polymerases add dNTPs to the 3' end);

and (d) identifying the sequencing reaction byproduct, thereby determining the nucleotide complementary to the base at said target position in each of the 10,000 DNA templates (see paragraph [0043]: "releasing PPi and generating detectable light" and "This process is repeated to generate a readout of the sequence of the target sequence").

With regard to claim 77, Chee teaches the limitations of claim 76, upon which claim 77 depends, as discussed in the rejection under 35 U.S.C. 102(e) above.

Chee does not teach that the reaction chambers have a center to center spacing of between 20 to 100 μm (claims 40, 52-55, 59-63 and 77). Chee does not teach reaction chambers having a width in at least on dimension of between 20 and 70 μm (claim 40).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention of the instant application was made to fabricate the wells in the planar substrate of Chee to achieve a center to center spacing of 20 to 100 microns. One would have been motivated to do so in view of Wolber, who teaches to space the features on a microarray at "less than or equal to two feature diameters, and preferably 1.5 to 2 feature diameters center to center spacing" (column 4, lines 20-25). Since the microspheres within the depressions in Chee's array are features on a microarray, and since Chee teaches microspheres ranging from 0.2 to 200 microns (for example, paragraph [0117]), one would have been motivated to follow the center to center spacing strategy suggested by Wolber because it would result in smaller and more compact arrays, which in turn would require less sample, allow for more rapid scanning, and be less expensive to manufacture (see Wolber, column 4, lines 15-29). A 1.5 to 2 feature diameter center to center spacing would correspond to 0.3 to 0.4 microns (for the smallest sized microspheres taught by Chee) to 300 to 400 microns (for the largest sized microspheres). This range of center to center spacing completely encompasses Applicant's range of 20 to 100 microns.

It would also have been *prima facie* obvious to one of ordinary skill in the art at the time the invention of the instant application was made to fabricate the wells in the

planar substrate of Chee to arrive at a width in at least one dimension of between between 20 and 70 μm, since Chee teaches bead sizes ranging from 0.2 to 200 μm (for example, paragraph [0117]). It would have been obvious to make the wells to correspond to the size of the beads (as illustrated in figure 1). Chee also teaches the surface of the substrate may be modified such that discrete sites are formed that can only have a single associated bead (paragraph [0110]) and that one means of retaining the beads in the sites on the array is compressive force (paragraph 0109]). These teachings, along with the illustration in figure 1, would have clearly suggested to one of ordinary skill in the art to make the dimensions of the wells or depressions correspond to the dimensions of the beads.

Claims 47-51 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chee et al (US 2003/0108867 cited reference A95 on the IDS of 8/2/2004) in view of Kambara et al (US 2001/0024790).

With regard to claim 47, Chee teaches *a method for carrying out separate parallel common reactions in an aqueous environment, comprising: (a) delivering a fluid containing at least one reagent to an array* (for example, see paragraph [0192]: "When sequencing templates are immobilized on beads and are associated with a substrate such as a fiber optic bundle, the fiber optic bundle can be contacted with different reagents, such as a well containing the nucleotide 'A'"),

wherein the array comprises a substrate comprising at least 10,000 discrete reaction chambers (see paragraph [0104]: "Arrays containing from about 2 different

nucleic acids (e.g. different beads, when beads are used) to many billions can be made..."),

each reaction chamber adapted to contain analytes (see for example paragraph [0112] ; the reaction chambers are "wells or small depressions" etched into the ends of the fibers of the fiber optic bundle),

and containing a starting material that is capable of reacting with the reagent (the starting material is the bead or microsphere with the attached "hybridization complex" (see for example paragraph [0009]) which is retained in the well or depression, i.e. reaction chamber, of the array (see for example paragraph [0109])).

With regard to claim 48, see paragraph [0192]: "...many individual reactions are performed in parallel on each array."

With regard to claim 49, see paragraph [0192]: Chee teaches contacting the fiber optic bundle array, which comprises immobilized nucleic acid sequences, with a well containing the nucleotide "A".

With regard to claim 50, see for example paragraph [0037]: "In addition to a first nucleotide, the solution also comprises an extension enzyme, generally a DNA polymerase."

With regard to claim 51, see paragraph [0192]: "This cycle can be repeated as necessary to generate a sequence of the sequencing template."

Chee does not teach reaction chambers having a volume of between 10 and 150 pL. Chee does not teach the reaction chambers are dimensioned such that when the fluid is delivered into each reaction chamber, the diffusion time for the reagent to diffuse

out of the well exceeds the time required for the starting material to react with the reagent to form a product. Chee does not teach washing the fluid from the array in the time period (i) after the starting material has reacted with the reagent to form a product in each reaction chamber but (ii) before the reagent delivered to any one reaction chamber has diffused out of that reaction chamber into any other reaction chamber.

Each of these limitations not expressly taught by Chee would have been obvious to one of ordinary skill in the art at the time the invention of the instant application was made for the following reasons:

and wherein the reaction chambers have a volume of between 10 to 150 pL: It would have been obvious to make the wells to correspond to the size of the beads (as illustrated in figure 1, and as suggested by Chee in paragraph [0112] for example: "The required depth of the wells will depend on the size of the beads to be added to the wells"). Since Chee teaches that the beads or microspheres of his invention range in size from about 0.2 microns (μm) to about 200 microns (see paragraph [0117]), and the volume of a sphere is $4\pi r^3/3$, it would have been obvious to make the wells or depressions ranging from 0.004 μm^3 for 0.2 μm beads to 4,190,476 μm^3 for 200 μm beads. Converting μm^3 to pL gives a range of 4×10^{-5} pL to 41,905 pL.

each of the reaction chambers being dimensioned such that when the fluid is delivered into each reaction chamber, the diffusion time for the reagent to diffuse out of the well exceeds the time required for the starting material to react with the reagent to form a product: It would have been obvious to design the reaction chambers such that the time required for the reagent to diffuse out of the reaction chamber exceeded the

time required for the reagent to react with the starting material, because otherwise the reagent would diffuse out of the well before completion of the reaction. Since Chee is concerned with pyrophosphate sequencing, and teaches that the PPi generated is directly proportional to the number of dNTPs incorporated (see paragraph [0041] for example), it would have been clear to one of ordinary skill in the art that the reaction must go to completion in order to derive the correct sequence. A complete reaction in pyrosequencing was known to be important to one of ordinary skill in the art as is evidenced by Kambara, who teaches of pyrophosphate sequencing: "If the reactions are not thoroughly carried out, reactions for each DNA chain proceed unevenly, which interferes with the base sequencing" last sentence, paragraph [0064].

and (b) washing the fluid from the array in the time period (i) after the starting material has reacted with the reagent to form a product in each reaction chamber but (ii) before the reagent delivered to any one reaction chamber has diffused out of that reaction chamber into any other reaction chamber. Chee clearly teaches washing after the reagent has reacted with the starting material. See for example paragraph [0044] where Chee teaches: "In a preferred embodiment a flow cell is used as a reaction chamber; following each reaction the unreacted dNTP is washed away and may be replaced with an additional dNTP to be examined".

While Chee does not teach washing *before the reagent delivered to any one reaction chamber has diffused out of that reaction chamber into any other reaction chamber*, it would have been obvious to do so, because it would have been obvious to wash before the PPi (pyrophosphate, a product of the reaction) diffused from one

reaction chamber to another in order to avoid detecting a false positive signal in a reaction chamber where no dNTP incorporation had occurred. Because pyrophosphate has a lower molecular weight than the reagent (i.e. the dNTP), pyrophosphate would diffuse more rapidly than the reagent. Therefore, one would have been motivated to wash before the pyrophosphate diffused from one reaction chamber to another, and in doing so one would have necessarily washed before the reagent (i.e. the dNTP) so diffused.

Claims 56-58 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chee et al (US 2003/0108867 cited reference A95 on the IDS of 8/2/2004) in view of Wolber et al (USPN 6,284,465, issued 9/4/2001, filed 4/15/1999) as applied to claim 54 above, and further in view of Kamb et al (US 2002/0172965).

The teachings of Chee and Wolber have been discussed above.

With regard to claim 56, these references do not teach that *at least 100,000 copies of a single species of nucleic acid template are immobilized on a plurality of the mobile solid supports.*

With regard to claim 57, Chee teaches that the sample DNA can be amplified (see second sentence, paragraph [0020]). Also, since there is no explicit definition of "picotiter plate" in the instant disclosure, the array of Chee qualifies as a "picotiter plate". Chee does not teach amplification to achieve *at least 2,000,000 copies* of the template.

With regard to claim 58, Chee teaches amplification by PCR (see paragraph [0019]: "the sample may be the products of an amplification reaction...such as PCR"). Chee does not teach amplification to achieve *at least 2,000,000 copies* of the template.

Kamb teaches a method of generating beads, each with a single species of oligonucleotide attached (paragraph [0027]). Kamb also teaches that the oligonucleotides are synthesized in such a way that each bead contains multiple copies of one oligonucleotide sequence, typically 10^6 to 10^{10} (see paragraph [0027]). This meets the limitation of claim 56.

Kamb also teaches that the oligonucleotides can be synthesized on the beads by way of a PCR reaction, in which each bead is coated with a specific primer pair and following the PCR reaction, one strand is released (see paragraph [0089]). Because Kamb stated that the method was designed to produce 10^6 to 10^{10} copies of a single oligonucleotide on each bead, this meets the limitation of claims 57 (*wherein each species of nucleic acid template is amplified to produce at least 2,000,000 copies per well of said nucleic acid template*) and 58 (*polymerase chain reaction*).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention of the instant application was made to produce the beads with a single species of oligonucleotide to use in the method of Chee by following the method for synthesizing such beads as disclosed by Kamb, since Chee teaches the target sequences may be prepared using "known techniques", and since Kamb demonstrates his method was a known, effective and efficient way for producing such beads. It would have been equally obvious to perform the bead-based PCR before or after the beads

were dispersed on the array. One of ordinary skill in the art would have easily appreciated that the beads produced by Kamb were structurally identical to the beads used in the method of Chee (compare figure 1 of Kamb with figure 1 of Chee), even though Kamb uses his beads in a different method.

Claim 64 is rejected under 35 U.S.C. 103(a) as being unpatentable over Chee et al (US 2003/0108867 cited reference A95 on the IDS of 8/2/2004) in view of Wolber et al (USPN 6,284,465, issued 9/4/2001, filed 4/15/1999) as applied to claim 63 above, and further in view of Nyren et al (WO 98/13523, cited reference B9 on the IDS of 8/2/2004).

The teachings of Chee and Wolber have been discussed above. These references do not teach using a dATP or ddATP analogue capable of acting as a substrate for a polymerase, but is incapable of acting as a substrate for a PPi detection enzyme.

Nyren teaches:

> "However, the PPi-based sequencing methods mentioned above are not without drawbacks. Firstly, it has been found that dATP used in the sequencing reaction (chain extension) interferes in the subsequent luciferase-based detection reaction, by acting as a substrate for the luciferase enzyme. In many circumstances, this interference severely limits the utility of the method." (page 2, penultimate paragraph, emphasis provided)
>
> "We now propose a novel modified PPi-based sequencing method in which these problems are addressed and which permits the sequencing reactions to be continuously monitored in real-time, with a signal being generated and detected, as each nucleotide is incorporated. This is achieved by using an dATP analogue, in place of dATP, which does not interfere with the luciferase reaction, and by performing the chain extension and detection, or signal-generation, reactions substantially simultaneously by including the "detection enzymes" in the chain extension reaction mixture." (page 3, first full paragraph, emphasis provided)
>
> "As will be described in the Example below, experiments have shown that substituting dATP with dATPaS allows efficient incorporation by the polymerase with a low

> background signal due to the absence of an interaction between dATPaS and luciferase. The signal to noise ratio is increased according to the present invention by using a nucleotide analogue in place of dATP, which eliminates the background caused by the ability of dATP to function as a substrate for luciferase. (page 6, last sentence continuing on page 7, emphasis provided)

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention of the instant application was made to practice the method suggested by the combined teachings of Chee and Wolber and substitute a dATP or ddATP analogue. The motivation to do so is clearly provided by the disclosure of Nyren, i.e. to overcome the problem of interference in the luciferase reaction.

Claim 72 is rejected under 35 U.S.C. 103(a) as being unpatentable over Chee et al (US 2003/0108867 cited reference A95 on the IDS of 8/2/2004) in view of Walt et al (USPN 6,023,540 cited reference A62 on the IDS of 8/2/2004).

Chee teaches *a method for sequencing a nucleic acid, the method comprising: (a) providing a plurality of single-stranded nucleic acid templates* (see paragraph [0024], where Chee teaches the nucleic acids may be either single or double stranded) *in an array having at least 50,000 discrete reaction sites* (see paragraph [0104]: "Arrays containing from about 2 different nucleic acids (e.g. different beads, when beads are used) to many billions can be made…");

(b) contacting the nucleic acid templates with reagents necessary to perform a pyrophosphate-based sequencing reaction coupled to light emission (see for example paragraph [0043]: "A substrate comprising microspheres containing the target sequences and extension primers, forming hybridization complexes, is dipped or

contacted with a volume (reaction chamber or well) comprising a single type of dNTP, an extension enzyme, and the reagents and enzymes necessary to detect PPi");

(c) detecting the light emitted from a plurality of reaction sites on respective portions of an optically sensitive device (see paragraph [0112]: "wells are made in a terminal or distal end of a fiber optic bundle comprising individual fibers...the cores of the individual fibers are etched"; see paragraph [0043]: "releasing PPi and generating detectable light" and "This process is repeated to generate a readout of the sequence of the target sequence"; and see paragraph [0192]: "...many individual reactions are performed in parallel on each array).

Chee does not expressly teach *(d) converting the light impinging upon each of said portions of said optically sensitive device into an electrical signal which is distinguishable from the signals from all of said other reaction sites; (e) determining the sequence of the nucleic acid templates based on light emission for each of said discrete reaction sites from the corresponding electrical signal.*

Walt teaches *(c) detecting the light emitted from a plurality of reaction sites on respective portions of an optically sensitive device* (column 11, lines 45-58: "Light returning from the distal end 212 of the bundle 202...is then imaged on a charge coupled device (CCD) camera 236");

(d) converting the light impinging upon each of said portions of said optically sensitive device into an electrical signal which is distinguishable from the signals from all of said other reaction sites (converting light into an electrical signal is the function of

a CCD camera; figures 8-10 clearly indicate distinguishable signals for different reaction sites).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention of the instant application was made to use a CCD camera (as taught by Walt) to detect the signals from the fiber optic array in the method of Chee, since Chee clearly desires parallel detection of reaction at individual sites on his fiber optic bundle array, and Walt demonstrates that a fiber optic bundle array connected to a CCD camera achieves this result. In fact, Walt teaches the same basic fiber optic bundle array as Chee, complete with microspheres residing in wells etched in the ends of the fibers (column 4, lines 4-8), and thus it would have been obvious to one of skill in the art to use a CCD camera with Chee's fiber optic bundle array. By using a CCD camera connected to the fiber optic bundle array of Chee, one of skill in the art would have arrived at the claimed invention, including the final limitation of *(e) determining the sequence of the nucleic acid templates based on light emission for each of said discrete reaction sites from the corresponding electrical signal.*

Claim 78 is rejected under 35 U.S.C. 103(a) as being unpatentable over Chee et al (US 2003/0108867 cited reference A95 on the IDS of 8/2/2004) in view of Nyren et al (WO 98/13523, cited reference B9 on the IDS of 8/2/2004).

Chee teaches the limitations of claim 76, upon which claim 78 depends, as discussed in the rejection under 35 U.S.C. 102(e) above. Chee does not specify that the fragments of nucleic acid are between 30 and 500 bases.

Nyren performs pyrophosphate sequencing of a fragment of nucleic acid immobilized on a bead. The fragment was 291 bases long (see page 22, third paragraph, and figure 5).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention of the instant application was made to use fragments within the range of 30 to 500 bases in the method of Chee, because Nyren demonstrates that this was a suitable size for pyrophosphate sequencing of bead-immobilized fragments of nucleic acid.

Claim 79 is rejected under 35 U.S.C. 103(a) as being unpatentable over Chee et al (US 2003/0108867 cited reference A95 on the IDS of 8/2/2004) in view of Nyren et al (WO 98/13523, cited reference B9 on the IDS of 8/2/2004) and further in view of Kamb et al (US 2002/0172965).

Chee teaches the limitations of claim 76, upon which claim 79 depends, as discussed in the rejection under 35 U.S.C. 102(e) above. Chee also teaches the target sequence may be amplified if needed (see paragraph [0020], second sentence). Chee does not specify that the amplification is performed *in the reaction chambers*. However, it would have been clearly obvious to one of skill in the art that when Chee teaches amplification of the target sample, it is intended this amplification occurs prior to the sequencing (hence the phrase "the target sequence is prepared" in paragraph [0020]).

Kamb teaches a method of generating beads, each with a single species of oligonucleotide attached (paragraph [0027]). Kamb also teaches that the

oligonucleotides can be synthesized on the beads by way of a PCR reaction, in which each bead is coated with a specific primer pair and, following the PCR reaction, one strand is released (see paragraph [0089]).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention of the instant application was made to produce the beads with a single species of oligonucleotide to use in the method of Chee by following the method for synthesizing such beads as disclosed by Kamb, since Chee teaches the target sequences may be prepared using "known techniques", and since Kamb demonstrates his method was a known, effective and efficient way for producing such beads. It would have been equally obvious to perform the bead-based PCR before or after the beads were dispersed on the array. One of ordinary skill in the art would have easily appreciated that the beads produced by Kamb were structurally identical to the beads used in the method of Chee (compare figure 1 of Kamb with figure 1 of Chee), even though Kamb uses his beads in a different method.

Allowable Subject Matter

Claims 1-9, 73-75 and 83 are allowable over the prior art.

The following is a statement of reasons for the indication of allowable subject matter: claims 1-9 and 73-75 disclose a novel method for sequencing a large number of nucleic acid templates simultaneously. The patentable feature lies in the unique method of preparing the multiple template nucleic acids, which involves forming a water-in-oil emulsion in which at least some water droplets ("aqueous microreactors") contain a single fragment of DNA, reagents necessary for amplification of the fragment, and a

single bead capable of binding the fragment and its amplification products. In this way, a collection of beads, each with many copies of a unique nucleic acid template, is prepared in a single tube.

While individual elements of this method were known in the prior art, there is no teaching, suggestion or motivation found in the prior art to combine these elements in such a way as to arrive at the claimed invention.

In assessing the prior art for claims 1-9 and 73-75, a priority date of 06/06/2003 was accorded based on the disclosure in provisional application 60/476952. The earlier provisional applications for which priority is claimed do not provide a disclosure of the invention of claims 1-9 and 73-75 satisfying the requirements of 35 U.S.C. 112. Provisional application 60/443471 makes no mention of a water-in-oil emulsion, and provisional application 60/465071, which is a copy of a research proposal, does not disclose dispersing the beads to an array "wherein a plurality of the reaction chambers comprise no more than a single bead".

With a priority date of 06/06/2003 for claims 1-9 and 73-75, the closest prior art to the claimed invention is the combination of Chee et al (US 2003/0108867 cited reference A95 on the IDS of 8/2/2004) and either Ghadessy et al (cited reference C25 on the IDS of 8/2/2004) or Nakano et al (24 April 2003). Chee describes parallel sequencing of nucleic acid fragments immobilized on beads on an array. However, Chee does not provide any teaching, suggestion or motivation to attach nucleic acids to beads using the emulsion/amplification technique described in claim 1. Ghadessy and Nakano each describe performing PCR reactions (amplification) in an emulsion format.

However, neither reference discloses or suggests including capture beads in the reaction so as to produce a collection of beads each bearing a single species of amplified nucleic acid. Consequently, neither reference discloses or suggests dispersing such beads on an array for sequencing.

Sepp et al (cited reference C83 on the IDS of 8/2/2004) discloses attaching nucleic acids to beads at a ratio to favor the attachment of a single molecule of nucleic acid each bead. The resulting beads were then emulsified to form "aqueous microreactors" containing reagents necessary for transcription and translation of the nucleic acid into protein, as well as a fluorescent substrate, such that beads attached to nucleic acids encoding an enzyme active on the substrate would be labeled with the fluorescent tag. The beads so labeled were then collected by flow cytometry. While this disclosure provides beads attached to nucleic acids in an emulsion, Sepp's purpose was entirely different from the sequencing technique described in claim 1. In addition, Sepp's method attaches nucleic acids to beads *before* creating an emulsion, whereas the method of claim 1 *utilizes* the emulsion to attach a single species of nucleic acid to each bead. It is unlikely that one of ordinary skill in the art, reading Sepp's disclosure would have been motivated to combine his teachings with the teachings of Chee and either Ghadessy or Nakano to arrive at the claimed invention.

Claim 83 is allowable over the prior art. The claimed invention is a novel method for obtaining sequence information for a nucleic acid molecule by using two or more primers in the same reaction. This strategy involves the use of two or more species of primers, where all but one species of primer are reversibly blocked, in a single reaction.

This strategy is possible in a sequencing-by-synthesis technique such as pyrophosphate sequencing, and allows one to obtain sequence from multiple locations on a template nucleic acid (e.g. from both ends of a double-stranded DNA molecule). The closest prior art is Nyren et al (cited reference C68 on the IDS of 8/2/2004), who suggests that sequential pyrophosphate sequencing reactions could be conducted on an immobilized template by carrying out the sequencing reaction with a first primer to determine a first sequence at a first site. The extended primer is then denatured from the immobilized template and wash away. A second primer is then annealed to a second site on the template to determine a second sequence and so on. The claimed invention is a novel approach in which multiple species of reversibly blocked primers are included in the reaction at the same time. A first primer is extended to obtain a first sequence. Further extension from the first primer is then prevented, and a second primer is unblocked to allow for its extension to produce a second sequence, and so on. Such a strategy is novel and non-obvious over the prior art.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Samuel Woolwine whose telephone number is (571) 272-1144. The examiner can normally be reached on Mon-Fri 9:00am-5:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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